

Epidemiologic issues in studies of association between apical periodontitis and systemic health

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Perhaps the most exciting issue currently facing the dental research community centers around a hypothesized connection between chronic inflammatory oral infections, most notably periodontal disease, and the development of adverse systemic health conditions. To date, inflammation of endodontic origin (i.e., apical periodontitis (AP)) has not been extensively studied in this regard despite being a commonly found sequel to bacterial infection of the dental pulp space. Although numerous differences exist between chronic inflammatory disease of periodontal and endodontic origins, there are some notable similarities, primarily that: (1) both often are associated with Gram-negative anaerobic bacteria, and (2) elevated cytokine levels may be released systemically from acute and chronic manifestations of both disease processes. This article provides a brief review of several important concepts concerning adverse general health outcomes as they relate to periodontal disease, summarizes recent epidemiologic studies of AP and root canal therapy, and reviews some general issues involved in the conduct of epidemiologic studies as well as how these issues apply to investigations that address potential links between endodontic inflammatory disease and adverse systemic health outcomes.

Introduction

Perhaps the most exciting issue currently facing the dental research community centers around a hypothesized connection between the presence of chronic oral infections and the development of adverse systemic health conditions. Several epidemiologic investigations have uncovered relationships between chronic periodontal disease and coronary heart disease, stroke, premature birth and/or low birth weight deliveries, and respiratory disease. The notion that chronic periodontal disease is linked with adverse systemic health outcomes is not universally accepted, as several reports assessing these relationships have reached alternative conclusions.

To date, inflammation of endodontic origin (i.e., apical periodontitis (AP)) has not received the same attention despite being a commonly found sequel to bacterial infection of the dental pulp space. AP can be acute and painful, but it also can be chronic and asymptomatic, and although AP is preventable or

treatable with root canal therapy (RCT), it may persist or recur after treatment is completed.

Despite numerous differences between chronic inflammatory disease of periodontal and endodontic origins, there are some notable similarities, primarily that: (1) both conditions share a common microbiota that often is associated with Gram-negative anaerobic bacteria, and (2) elevated cytokine levels may be released systemically from acute and chronic manifestations of both disease processes (e.g., increased concentrations of inflammatory mediators have been detected both in the gingival crevicular fluid of subjects with periodontal disease and in the periapical tissues of endodontically involved teeth). If current investigations into links between chronic periodontal inflammation and adverse general health outcomes ultimately suggest positive (or especially causal) associations, a similar attempt to link apical inflammatory lesions with general health outcomes cannot be far behind, but to date, especially compared with the multitude of published research efforts involving cytokines and

inflammatory mediators in periodontal disease, the study of these molecular markers in endodontic disease has not been as extensively documented. Because little information about these relationships currently exists, patients and practitioners lack potentially important knowledge about risks that patients may run by keeping teeth with chronic, asymptomatic AP.

The purposes of this article are threefold: (i) to provide a brief review of some important concepts concerning adverse general health outcomes related to periodontal disease; (ii) to summarize recent epidemiologic studies of AP and RCT; and (iii) to review some of the more important issues involved in the conduct of epidemiologic studies in general, and specifically how these issues apply to investigations that address potential links between endodontic inflammatory disease and adverse systemic health outcomes.

AP and systemic health: outcomes of interest

As stated above, numerous epidemiologic studies have found associations between periodontal disease and cardiovascular diseases (CVD), leading researchers to propose that in response to Gram-negative anaerobic bacterial endotoxins found in periodontal disease, certain individuals produce an overabundance of localized inflammatory cells that produce cytokines, which then are released into the systemic circulation and ultimately may contribute to vascular damage and cardiovascular events. Although systemic levels of inflammatory mediators have been measured in endodontic patients (1, 2), little exploration of potential links between inflammation of endodontic origin and CVD has been done, and it is not unreasonable to expect connections similar to those reported in the periodontal literature, given the predominance of Gram-negative anaerobes associated with endodontic infections and the evidence of cytokine production in inflamed pulp and periapical granulomatous tissues.

It is beyond the scope of this article to describe the microbiology and immunology of endodontic disease (these topics are covered in detail elsewhere), but if relationships between apical inflammatory disease and general health are to be the focus of scientific inquiry, what general health outcomes should be investigated? It makes sense to start with the two groups of outcomes that have been at the center of most periodontal

disease–systemic disease studies: CVD and adverse pregnancy outcomes.

CVD

CVD, including coronary heart disease, stroke, and peripheral vascular diseases, are far and away the greatest cause of death and disability in the US, with 455 000 men and 505 000 women dying of the conditions in 1995 (3). Globally, although heart disease and stroke were rated fifth and sixth by the World Health Organization in terms of disability in 1990, they are expected to rank first and fourth by the year 2020 (4, 5). The financial burden of CVD is staggering, with estimated US costs for medical expenses and lost productivity in the neighborhood of \$274 billion (3). Clearly, the human and economic costs of CVD are overwhelming, both nationally and internationally.

An inflammatory component to arteriosclerosis has long been suspected (6), but the connection between oral infection and CVD only recently has come to the forefront of epidemiologic research. In the late 1980s, Mattila et al. (7–10) suggested a relationship between overall poor dental health and atherosclerosis/myocardial infarction. The independent variable in these early studies, the ‘Total Dental Index’, was a summary measure of oral health that included aspects of endodontic disease in addition to dental caries and periodontal disease. This pioneering Scandinavian research was followed up by US investigators, who confirmed a relationship between periodontal disease and coronary heart disease (11).

Based on these findings, Beck et al. (12) proposed a theoretical model relating chronic periodontal infections to the development of atherosclerosis and thromboembolic events. The postulated biologic mechanism rests on the notion of an underlying hyperinflammatory response trait in the host that places him/her at risk for developing both periodontal disease and atherosclerosis. In the hypothesized chain of events, susceptible individuals produce a hyperinflammatory response to periodontal bacteria and their associated endotoxins. Once periodontal disease is established, bacterial endotoxins induce the host to overproduce cytokines such as interleukin (IL)-1 β and tumor necrosis factor- α , which then are released systemically. Production of the cytokine IL-6, among

others, can lead to hepatic generation of acute phase reactants such as C-reactive protein (13), which has been linked to atherogenesis and thromboembolic events (14). Thus, the hyperinflammatory response leads to both more serious periodontal disease and greater risk for CVD.

To test this hypothesis, the investigators (12) conducted a cohort study in a sample of Boston area men and found that new coronary heart disease and stroke events were more common among subjects with high mean alveolar bone loss scores than among those with low scores, suggesting a positive (but not necessarily causal) association between periodontal disease and CVD in this sample. Research aimed at lending credence to this hypothesized mechanism is ongoing, and includes the recently published finding that levels of C-reactive protein were higher in the systemic circulation of individuals with chronic periodontal disease than those without periodontal disease in a random sample of US adults (15) and in the ARIC study population (16). Other investigations also have found significant relationships between chronic periodontal disease and both coronary heart disease (17, 18) and stroke (19–21). However, these connections are not universally accepted, as several reports assessing these relationships have reached alternative conclusions (22, 23).

Adverse pregnancy outcomes

Adverse pregnancy outcomes are the other major classification of conditions identified as being potentially related to (or in part caused by) chronic periodontal infections. Two perinatal outcomes in particular, pre-term delivery and low birth weight, have been most commonly used as endpoints in this body of literature. In the US in 2002, 7.8% of babies ($n = 314\,077$) were born with low birth weight, and 12.1% ($n = 480\,812$) were born pre-term (24). Like CVD, adverse pregnancy outcomes unfortunately are quite common and are associated with an overwhelming burden of morbidity and mortality.

As dependent variables, both low birth weight and pre-term delivery theoretically can be assessed as continuous outcomes, but in most publications they are analyzed as binary variables, e.g., births at <37 weeks of gestation representing pre-term delivery and babies weighing <2500 g at birth being classified as

low birth weight (25, 26). Among the biologic mechanisms proposed to explain links between periodontal disease and adverse pregnancy outcomes are that periodontal pathogens disseminate systemically within the mother and gain access to the fetal compartment, disturbing normal cytokine and hormone-regulated gestation (26, 27).

Just as in the CVD literature, there are conflicting reports as to the presence and/or strength of links between chronic periodontal disease and adverse pregnancy outcomes. While some studies identify significant associations (28, 29), others do not (25). In one recent systematic review (30), the authors analyzed six case-control, three cross-sectional or longitudinal, and three intervention studies that met their inclusion criteria, concluding that while chronic periodontal disease may be a risk factor for adverse pregnancy outcomes, there was not enough evidence to support an inference of causality, and that intervention studies would be necessary to have confidence in such an assertion.

If CVD and adverse pregnancy outcomes are selected as general health outcomes of interest to the endodontic research community, investigators should realize that longitudinal studies addressing CVD will tend to be more costly and lengthy than those ascertaining adverse pregnancy outcomes, simply because prospective studies focusing on adverse pregnancy outcomes would take each subject no longer than about 9 months to complete, while CVD outcomes potentially can take years to develop. One way in which periodontal researchers have examined CVD outcomes is by using surrogate endpoints, i.e., endpoints occurring more rapidly than more distal outcomes of interest but thought to be able to provide useful knowledge about the likelihood of those distal outcomes. One example of a surrogate endpoint familiar to dentists is the loss of periodontal attachment, which often is considered a surrogate endpoint for tooth loss (31). In the periodontal disease–CVD literature, surrogate outcomes for clinical CVD events have included coronary artery intima-media wall thickness (a marker of the pathological process of atherosclerosis and a pre-clinical indicator of atheroma formation) (32) and radiopacities in the vicinity of C3–C4 on panoramic radiographs (radiographs being a non-invasive method for assessing carotid artery stenosis) (33). If surrogate endpoints are selected as study outcomes by the AP research community, their hypothesized relationships

with the outcomes of ultimate interest should be thoroughly understood (34).

Endodontic and epidemiologic terminology

To critically evaluate epidemiologic studies of AP and RCT, one must first be familiar with some basic terminology used in the relevant bodies of literature:

- As most readers are aware, AP is defined as ‘an acute or chronic inflammatory lesion around the apex of a tooth caused by bacterial infection of the pulp canal system’ (35). Clinical diagnosis of AP requires assessment of the patient’s symptoms, signs, and radiographs. Histologically, AP is represented by a periapical inflammatory response (often termed periapical granuloma) that arises after resorption of adjacent supporting bone and local infiltration of inflammatory cells (36). Chronic periapical inflammation occurs when a balance is established between the local tissue response and the bacterial elements emerging from the root canal system. The radiographic appearance of AP usually is a radio-lucent area of variable size at the apex of the affected root, although occasionally, radio-opaque zones can be seen (36). Deep caries lesions (and/or their subsequent restoration) surely are the most common predecessors to pulpal disease, although trauma, dental procedures, and some congenital anomalies also can be responsible for its development. AP is a sequel to bacterial infection of the dental pulp space and can be treated (or prevented) by elimination of bacteria from the roots via RCT.
- *Epidemiology*: The study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to control of health problems (37).
- *Population (when designing a sample)*: The whole collection of units from which a sample may be drawn; not necessarily a population of persons; the units may be institutions, records, or events. The sample is intended to give results that are representative of the whole population (37).
- *Prevalence, or prevalence rate*: The total number of all individuals who have an attribute or disease at a particular time . . . divided by the population at risk of having the attribute or disease at that point in

time (37). For example, if 32 out of 1000 dentate people had chronic AP at a given point in time, the prevalence of chronic AP would be 3.2%.

- *Incidence, or incidence rate*: The rate at which new events occur in a population . . . The numerator is the number of new events that occur in a defined period; the denominator is the population at risk of experiencing the event during this period, sometimes expressed as person-time (37). For example, if 32 people out of 1000 developed AP during a 2-year interval, the incidence of AP could be expressed as 16 cases per 1000 person-years.
- *Exposure, or exposure variable*: The putative cause (in the comparison of a putative cause and its effect) (38). These terms are synonymous with the terms ‘independent variable’ and ‘predictor’, and may be psychological, behavioral, biological, or genetic variables.
- *Outcome, or outcome variable*: The hypothesized effect (in the comparison of a putative cause and its effect) (38). These terms are synonymous with the terms ‘dependent variable’ and ‘response’, and are generally health-related variables.

Epidemiology of AP and RCT

In general, prevalence studies are more common than incidence studies because by definition, incidence studies must collect information on subjects at more than one time point so that rates of new disease during a given period can be calculated. This implies greater complexity and cost compared with prevalence studies, which require subjects to be examined at only one visit. To my knowledge there are no published epidemiologic studies for which the incidence of AP is the primary focus, and only one for which incidence of endodontic procedures is the primary focus: Boykin et al. (39) reported that during a 2-year period in a representative community sample of 873 dentate adults at least 45 years of age, 13% of study participants received at least one endodontic procedure, and endodontic procedures comprised about 2% of the total number of dental procedures completed. Conventional orthograde RCT accounted for 94% of the endodontic procedures, with endodontic retreatments and apicoectomies constituting the remainder.

The body of scientific literature reporting the prevalence of AP and/or root-filled teeth can be

separated into two mutually exclusive categories: 'Community-based' and 'Clinic-based' studies. Samples for community-based studies are comprised of individuals drawn at random from among the population of residents of a given region or area, usually by using voting rosters or other governmental lists, while those for clinic-based studies are drawn from among patient populations of hospitals, dental schools, public health clinics, or private practices. This distinction is important because in general, community members at large (subjects) might differ substantially from those seeking care (patients) with respect to socio-economic status, attitudinal and/or behavioral characteristics, and health status (40).

Tables 1 and 2 summarize the community- and clinic-based prevalence studies of AP and root-filled teeth published since 1986 in English-language journals to which this author had access. The articles were identified via two methods: (1) using PubMed, a publicly available database created by the US National Library of Medicine, searching the keywords 'prevalence' and 'root canal'; and (2) perusing review tables presented in some of the cited articles to uncover other articles not identified via the PubMed search. Tables 1 and 2 provide a relatively thorough set of articles on which to base broad generalizations, but there is no guarantee that all relevant articles are listed. Several related types of studies that do not appear in the tables include those that:

- reported data only for root-filled teeth and not for AP (41),
- enrolled only people with at least one root-filled tooth (42),
- examined only crowned teeth (43) or root-filled teeth (44),
- included only patients or subjects who had been selected for the study because they reached a threshold level of anticipated treatment costs (45) or because they belonged to a group of regular dental attenders (46).

Most of the listed studies were carried out in Western European countries. Research of this type in the US has thus far been rare, partially because diagnosis of AP requires radiographs and exposure to radiation solely for research (rather than diagnostic or therapeutic) purposes poses certain ethical questions (47, 48).

Tables 1 and 2 are in the same format, and present the published reference, the country in which the study was conducted, the age (range) of subjects, and the number of subjects (and teeth) studied, followed by the:

- percent of subjects with ≥ 1 tooth with AP,
- percent of all teeth with AP,
- percent of root-filled teeth with AP,
- percent of non-root-filled teeth with AP,
- percent of all subjects with ≥ 1 root-filled tooth,
- percent of all teeth with root fillings,
- percent of root-filled teeth with 'inadequate' RCT according to that study's criteria (generally based on radiographic density of the root filling material and/or its extension relative to the tooth's radiographic apex),
- ratio of root filled in all teeth: AP in all teeth,
- ratio of AP in root-filled teeth: AP in non-root-filled teeth.

In any compilation of epidemiologic investigations that address a single research topic, one should expect a variety of findings because of differences across studies with respect to case definitions, measurement techniques, and population characteristics. Despite this expected variation, the following conclusions can be drawn based on the listed prevalence studies of AP and root-filled teeth.

AP and root-filled teeth are relatively common conditions

Tables 1 and 2 show that AP occurred in 14–70% of all subjects and 0.6–8.5% of all teeth, while root-filled teeth were evident in 22–78% of subjects and 1.3–21.5% of teeth, respectively. Figures 1 and 2 present information from Tables 1 and 2; each data point in each figure represents information provided by one of the listed studies. In both figures the mean number of teeth per dentate subject is on the x -axis. In Fig. 1 the y -axis represents the percent of all teeth with AP, while in Fig. 2 the y -axis represents the percent of all teeth that were root filled. The mean number of teeth per subject most likely is well correlated with the mean age of the sample, but this information could not be verified for most studies because the predominant way most investigators reported age data was according to the number of people within each age group classification (e.g., 23% of subjects were from 20 to 29 years old). However, all studies reported the number of subjects

Table 1. Community-based prevalence studies of apical periodontitis (AP) and root-filled (RF) teeth*

Reference	Country	Age (years)	Subjects (teeth)	Mean teeth per subject	Prevalence of AP (%)			Prevalence of RF teeth (%)				Prevalence ratios	
					Subjects with ≥ 1 teeth	In all teeth	In RF teeth	In non-RF teeth	Subjects with ≥ 1 teeth	In all teeth	Inadequate RCT	RF in all teeth/AP in all teeth	AP in RF teeth/AP in non-RF teeth
(59)	Norway	35	141 (3917)	{27.8}	30	1.4	{25.6}	{0.53}	53	3.4	NRC†	{2.4}	{48.3}
(107)	Sweden	≥ 20	743 (17 430)	{23.5}	NRC	2.9	24.5	{0.87}	NRC	8.6	70	{3.0}	{28.2}
(58)	Norway	50	119 (2940)	{24.7}	NRC	3.5	44.0	{1.4}	56	{5.9}	NRC	{1.7}	{31.4}
(57)	Switzerland	66	143 (2004)	{14.0}	NRC	8.5	31.0	{2.8}	78	20.3	64	{2.4}	{11.1}
(108)	Norway	35	118 (3282)	{27.8}	14	0.6	{38.1}	{0.06}	24	1.3	NRC	{2.2}	{635.0}
(67)	Finland	76, 81, 86	169 (2355)	{13.9}	{41}	{7.1}	{16.7}	{4.4}	{78}	{21.5}	{75}	{3.0}	{3.8}
(53)	Portugal	30–39	179 (4446)	{24.8}	26	2.0	22.0	{1.7}	22	1.5	54	{0.8}	{12.9}
(50)	Lithuania	35–44	147 (3892)	{26.5}	70	7.2	{35.6}	{1.5}	72	8.2	{88}	{1.1}	{23.7}
(63)	Denmark	{22–63}	614 (15 984)	{26.0}	NRC	3.4	52.2	0.9	52	4.8	NRC	{1.4}	{58.0}

*Numbers in { } calculated by the present author from information published in the article.

†NRC, not reported or calculable from information presented in the article.

Table 2. Clinic-based prevalence studies of apical periodontitis (AP) and root-filled (RF) teeth*

Reference	Country	Age (years)	Subjects (teeth)	Mean teeth per subject	Prevalence of AP (%)			Prevalence of RF teeth (%)			Prevalence ratios		
					Subjects with ≥ 1	In all teeth	In RF teeth	In non-RF teeth	Subjects with ≥ 1	In all teeth	Inadequate RCT	RF in all teeth/AP in all teeth	AP in RF teeth/non-RF teeth
(109)	Sweden	≥20	200 (4889)	24.4	{63}*	5.2	26.4	{2.0}	{84}	13.0	{44r}	{2.5}	{13.2}
(110)	The Netherlands	≥20	184 (4196)	{22.8}	45	6.0	39.3	5.2	NRC	2.3	51	{0.4}	{7.6}
(56)	US	≥18	208 (5272)	{25.3}	NRC	4.1	31.3	{3.4}	NRC	5.5	58	{1.3}	{9.2}
(111)	Scotland	≥20	340 (8420)	{24.8}	{68}	{4.8}	{51.7}	2.0	{55}	5.6	{58r}	{1.2}	{25.8}
(112)	Germany	≥12	323 (7897)	{24.4}	NRC	3.0	61.0	{1.4}	NRC	2.7	86	{0.9}	{43.6}
(66)	Belgium	≥18	206 (4617)	{22.4}	63	6.6	40.4	{4.1}	NRC	6.8	{59}	{1.0}	{9.8}
(52)	France	≥18	208 (5373)	{25.8}	NRC	7.4	29.7	2.0	NRC	19.1	79r	{2.6}	{14.8}
(51)	France	≥21	344 (7561)	{22.0}	NRC	7.3	31.5	1.7	NRC	18.9	{69}	{2.6}	{18.5}
(55)	Canada (Toronto)	25-40	400 (10 474)	{26.2}	NRC	{3.7}	{44.3}	{2.4}	38.7	3.0	NRC	{0.8}	{18.4}
(64)	Spain	≥18	180 (4453)	{24.7}	61	{4.2}	{64.5}	{2.9}	41	{2.1}	NRC	{0.5}	{22.2}

*Numbers in { } calculated by the present author from information published in the article.
NRC, not reported or calculable from information presented in the article; r, roots.

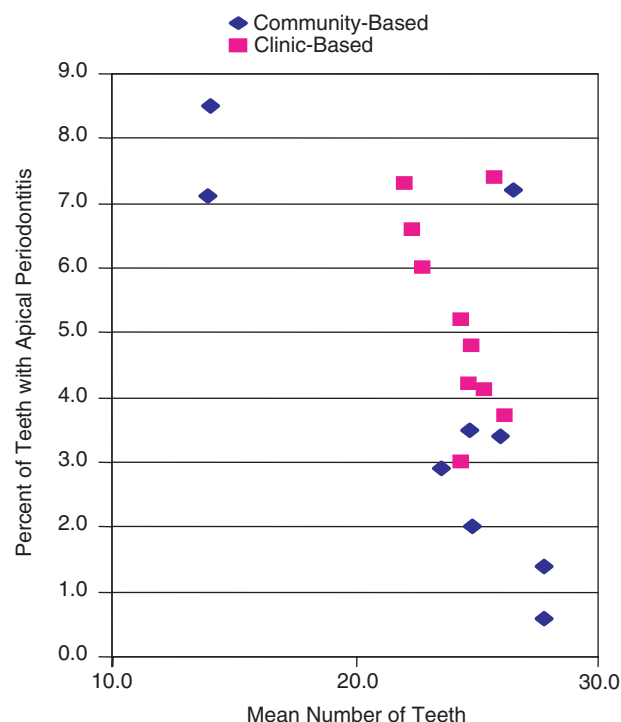


Fig. 1. Percentage of teeth with apical periodontitis, by study setting.

and total number of teeth, so mean number of teeth per subject was readily calculable but not always presented.

One must use caution when comparing information across multiple studies carried out in different settings, various countries, and different populations, but as long as the potential implications of these differences are appreciated, such comparisons can be helpful in understanding trends. In the figures, the overall pattern of data points parallels a general pattern found in each study, namely that within each sample, the percent of teeth with AP and root-filled teeth tended to decrease with an increasing mean number of teeth. Because the mean number of teeth tends to decrease with increasing age, if age were plotted on the x -axis as in Eriksen (49), age would be directly correlated with the percent of teeth with AP and the percent of all teeth with root fillings.

A more interesting finding is that, except for one Eastern European study with an extraordinarily high prevalence of AP (50), clinic-based studies tended to report higher percentages of teeth with AP, holding the mean number of teeth constant (Fig. 1). Contrast this with the prevalence of root-filled teeth, which (except for two French studies that report an extraordinarily high prevalence of root-filled teeth (51, 52)) shows no clear pattern emerging between the two study settings (Fig. 2). One hypothesis for this finding is that to be ‘at

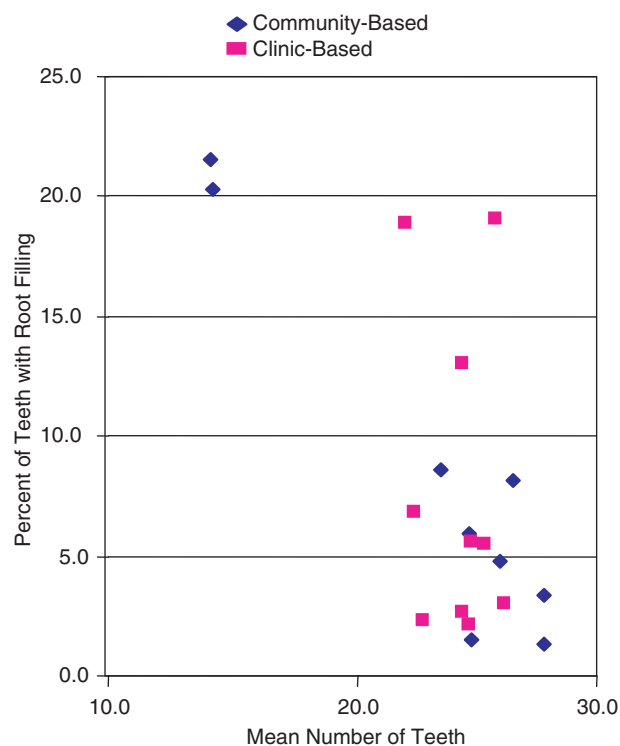


Fig. 2. Percentage of teeth with root filling, by study setting.

risk’, or ‘eligible to have’ AP or RCT, a tooth must be present. This statement may seem obvious but it is likely that in populations with relatively limited resources or access to care, subjects might have more teeth affected by AP simply because they cannot afford to visit the dentist for extraction of teeth that warrant removal, especially asymptomatic teeth, which are precisely the type of teeth that would be seen on radiographs in an investigation of AP. It thus is not surprising that the clinic-based studies, which correspond to settings that tend to serve patients of more limited means, report a higher prevalence of teeth with AP, which further serves to illustrate the difference between community and clinic samples. Conversely, RCT is not a disease but a treatment; it costs money and must be provided by practitioners with certain training, skills, and equipment. Because clinic-based studies include patients who presented for care, there might be a tendency for these groups to be more equivalent to community-based populations with respect to prevalence of root-filled teeth than AP prevalence. In other words, clinic-based populations might have a lesser overall economic status but at least have an interest in receiving care, and this may achieve a balance against community-based populations, which might have a

greater overall economic status but comprise a cross-section of the population that contains a wide range of interest in dental care.

As seen in Fig. 1, the only community-based study that rivals the clinic-based studies in terms of AP prevalence was conducted in Lithuania (50). It is likely that access to care in this country is relatively poorer than in the other studies, which were carried out in Scandinavia, Western Europe, or the US, countries that likely have relatively greater access to care. This point is further illustrated by this study's prevalence ratio (i.e., root-filled teeth: teeth with AP) of only 1.1, compared with ratios for most of the other community-based studies (Table 1). This ratio can be used as a general guideline to represent the relative amount of access to endodontic care in a population relative to its amount of endodontic disease. The clinic-based studies (except for the two aforementioned French studies) tended to report lower values for this ratio than those of the community-based studies, again indicating that subjects in clinic-based studies tended to have lesser access to care, lesser means, or lower health education than subjects in community-based studies, holding the mean number of teeth constant. One exception is a study from Portugal (53) where this ratio was low, primarily because of the reported prevalence of root-filled teeth being relatively low.

Finally, it must be remembered that root-filled teeth and AP occur secondarily as a direct or indirect result of dental caries, i.e., through direct involvement of the pulp through caries or via insult to the pulp via subsequent restoration of the tooth. Thus, in populations with low caries experience, one would tend to find fewer teeth with AP and fewer root-filled teeth, all other things being equal.

AP is much more common among root-filled teeth

The last column in the tables shows that the prevalence ratio (i.e., prevalence of AP in root-filled teeth divided by prevalence of AP in non-root-filled teeth) ranges from a low of 3.8 to a high of 635.0, indicating that at the very least, AP is about four times as common in root-filled teeth as in non-root-filled teeth. Consistent with this view are findings from a study by Kirkevang & Wenzel (54) showing that 'the most important risk

indicator of having AP in the individual was the radiographic evidence of root fillings.'

The overall quality of RCT has generally been cited as poor, with inadequate fillings reported in 44–86% of root-filled teeth or roots (see tables; also reviewed in (55)). While RCT designated as inadequate by specific investigators does not necessarily correspond to unsatisfactory endodontic results, several authors have suggested that poorer quality RCT is more frequently associated with AP (56–60).

Epidemiologic issues

There are several basic issues related to study protocol that all research investigations must address, and epidemiologic studies are no exception. Certain common themes (e.g., measurement error, potential for bias) must be understood and appreciated in advance so that studies can be planned properly. This section highlights some of the many important issues that should be considered by investigators when planning studies focusing on the assessment of teeth with AP and/or root-filled teeth.

Study design issues

Causal vs. non-causal relationships

Epidemiologic studies, by definition, use people as subjects; *in vitro* or animal experiments, while important components in the quest for causal inference, do not fall under the umbrella of epidemiologic research and are not discussed here. Among the many designs employed in epidemiologic studies, not all are equally capable of elucidating a cause-and-effect relationship between an exposure and an outcome. Bradford-Hill (61), in a classic article, outlined several qualities of epidemiologic research studies that affect our ability to infer causal relationships from research findings.

As a general rule, among large-scale study designs, confidence in assuming a cause-and-effect relationship from cross-sectional studies is weakest, followed by case-control studies, then observational cohort studies, and finally by randomized controlled trials, which provide the best evidence for interpreting causation (62). Because cross-sectional studies examine data only at one point in time, if two conditions are noted simultaneously, one cannot determine whether one condition occurred before the other or if both occurred

simultaneously. Case-control and observational cohort studies are longitudinal in nature and thus can address the issue of temporality between the independent variable and the outcome, but these designs are prone to various types of bias that arise from the fact that subjects essentially 'self-select' their own exposure groups (e.g., smokers vs. non-smokers). Randomized controlled trials offer the best evidence for causal relationships because they are longitudinal and because they assign subjects to treatments at random, thus minimizing the potential for bias induced by subject self-selection. Unfortunately, such trials tend to be more time-consuming and costly than studies using other designs, and also can present more ethical dilemmas because of subjects' having to be assigned to specific interventions.

With regard to root-filled teeth and/or AP, depending on the study design, it may be impossible to determine whether AP was present, or RCT was conducted, prior to development of a systemic condition like CVD. In cross-sectional studies for example, it would be impossible to determine from a single radiographic image whether for a given tooth, AP and/or RCT occurred prior to development of the outcome of interest. This problem may be easier to address in studies where an adverse pregnancy outcome is the dependent variable, because these outcomes would occur over a shorter period of time than most CVDs (i.e., about 9 months), and thus cohort studies might be more feasible.

Case definitions of AP across studies

The fact that different studies employ different definitions of AP is not newsworthy, but still it deserves mention as a potential reason for discrepancies across studies. Among the many definitions used to define AP are the following:

- Any tooth with periapical index score > 2 (50, 52, 55, 63, 64). The periapical index is a five-point scale designed to help correlate radiographic assessments of endodontically treated and non-treated teeth with a gold standard, histologic evaluation of a designated set of five 'representative' histologic situations (65). Examiners are calibrated by being asked 'Which of these five sets of standardized photographs does your test radiographic material most resemble?' On this five-point scale, scores 1

and 2 are usually considered 'healthy' for the purposes of epidemiologic evaluation, while scores 3–5 are considered 'unhealthy' (i.e., AP).

- Widening of the periodontal ligament (widening of the apical part of the periodontal ligament not exceeding two times the width of the periodontal ligament space) or periapical radiolucency (radiolucency in connection with the apical part of the root, exceeding at least two times the width of the lateral part of the periodontal ligament) (66).
- A clearly visible apical radiolucency contrasting with the physiological periodontal membrane (57) or a clearly discernible local widening of the apical periodontal membrane space (67).

Many times in studies evaluating the efficacy of endodontic treatment, AP is not defined explicitly; rather, 'endodontic success' is defined, and one must infer that AP is the opposite of endodontic success. Some examples of 'successful' endodontic therapy are where one observes radiographically:

- Normal periapex (if pre-operatively normal) or healing of radiolucent area or clear reduction in size of lucency (if pre-operative radiolucency evident) (68).
- Tooth with normal contours, width, and structure of the periodontal margin, or contours widened mainly around an excess of filling material (69).
- Apical periodontal ligament space not more than double its width in other areas of the root (70).
- Normal periapical bone and periodontal ligament structures, absence of periapical pathosis, and apical periodontal ligament no wider than twice the width of the rest of the periodontal ligament (71).

Alternatively, the presence of AP can be scored on a continuum as determined subjectively, e.g., where examiners rate periapical destruction of bone as 'definitely not present', 'probably not present', 'unsure', 'probably present', or 'definitely present' (72).

It is unrealistic to think that all researchers (and clinicians) in the field of endodontics could come to agreement on a uniform definition of AP. Still, it must be recognized that as long as different case definitions are applied, it remains a possibility that at least part of the variation across studies could result from variations in the case definition of AP. This is true not only for traditional endodontic studies in which AP is the outcome of interest, but also for investigations of the

AP/systemic disease relationship, where AP is the independent variable.

Estimating the burden of endodontic inflammation

In the periodontal literature, investigators have primarily been concerned with chronic, rather than acute, inflammatory disease, because periodontal disease primarily is a chronic condition, although acute abscesses are possible. Proposed biologic mechanisms explaining a link between periodontal and systemic diseases generally involve chronic exposure of systemic tissues to periodontal pathogens, thus investigators in this area generally have chosen clinical attachment level as the variable to best represent the cumulative, historical ‘burden’ of chronic periodontal disease for individual subjects at each clinical examination.

Clinical attachment level is defined as the distance (in millimeters) from the cemento-enamel junction (CEJ) to the base of the periodontal pocket at a given site, and is calculated indirectly by using a periodontal probe to measure the distance from the CEJ to the gingival crest (recession) and the distance from the gingival crest to the base of the periodontal pocket (pocket depth). Clinical attachment level is a historical measure of periodontal disease on existing teeth, i.e., it is not a snapshot of active periodontal disease at the time of assessment, but rather a cumulative measure over the life of each existing tooth. Using site-specific assessment at two, three, or six sites around each tooth (73–75), summary measures can be calculated for each individual to describe his/her relative amount of periodontal disease experience.

By comparison, endodontic disease can manifest as an acute or chronic condition, and depending on whether involved teeth have been treated endodontically, questions could be raised regarding how to ‘count’ the historical, cumulative burden of endodontic inflammation incurred by an individual. Additionally, if the outcome is a condition like prevalent coronary heart disease (which can result from a lifetime of insult to cardiac vasculature), then a cumulative measure of endodontic exposure is reasonable; however, if the outcome is a biologic marker like C-reactive protein or IL-6, or even a recent event like myocardial infarction or stroke, then a contemporaneous measure of the exposure may be more relevant. Several methods for estimating the ‘burden of endodontic infection’ are

proposed below, along with the major advantages and disadvantages of each method.

For cross-sectional studies

Method 1: Determine whether the subject has any teeth with AP, and use this as a binary variable (i.e., ‘Does the subject currently have AP, yes or no?’).

- *Advantages:* Easily understood; if at least one ‘obvious’ AP lesion exists, ‘borderline’ calls on other teeth would not be important.
- *Disadvantages:* Estimated burden of endodontic inflammation would be identical for subjects with one AP lesion or multiple AP lesions.

Method 2: Count the number of teeth with AP and use this as an integer variable.

- *Advantage:* Allows for a ‘dose–response’ relationship to be investigated.
- *Disadvantage:* The effect of ‘borderline’ calls on the burden of endodontic inflammation estimate could be substantial.

For longitudinal studies

Method 1: At baseline, count the number of teeth with AP. At each subsequent visit, add to that number a count of any additional teeth that experienced a new AP lesion between baseline and that visit, and continue this strategy for each subsequent visit. This method would provide a number ranging from 0 to 32 at each visit, and would be a monotonic, cumulative measure analogous to the Decayed, Missing, Filled Teeth index used in quantifying caries experience (47).

- *Advantage:* Allows the estimated burden of endodontic inflammation to change over time.
- *Disadvantage:* Once a tooth has AP it continues to contribute value to the cumulative figure and thus does not allow for teeth with successful RCT to stop contributing to the burden of endodontic inflammation calculation.

Method 2: At each visit, determine whether each tooth has AP, and use that integer value as a time-varying covariate in the statistical analyses.

- *Advantage:* Allows for positive or negative change in the number of teeth affected at any single visit.
- *Disadvantage:* Requires a more sophisticated statistical analysis and thus more involved biostatistical assistance.

Method 3: At baseline, determine which teeth have AP, and at each subsequent visit determine whether these teeth still have AP. From the visit dates and the

presence or absence of AP, calculate a value of ‘AP years’ for each tooth, and sum over all teeth within an individual. This method would continue at each successive visit.

- *Advantage:* Allows for a contribution of ‘time with AP’ for each tooth within an individual.
- *Disadvantages:* Relatively difficult to compute; requires algorithms for teeth with AP at one visit that is not present at a subsequent visit (e.g., extraction, successful endodontic treatment); affected by the chosen follow-up interval (i.e., shorter follow-up intervals permit more valid imputation of the dates on which changes in a tooth’s AP or root-filled status occur, because the dates of changes occurring during a short period of time, on average, would be more accurately estimated than the dates of changes occurring during a long period of time (76).

Investigators estimating burden of endodontic inflammation within individuals face additional questions, including but not limited to the following:

1. *Lesion size:* Should a 4 mm AP lesion and a 10 mm AP lesion be considered to contribute the same burden of endodontic inflammation?
2. *Roots vs. teeth:* Should a three-rooted tooth with an AP lesion on only one root be assigned the same burden of endodontic inflammation as that same tooth with an AP lesion on each root?
3. *Microbial composition:* Given the literature reporting differences in microbiologic composition between initial and refractory AP lesions (e.g., (77)), and the potentially different local and/or systemic effects posed by different bacterial strains, should endodontically treated teeth with AP lesions be assigned the same burden of endodontic inflammation as non-endodontically treated teeth with similar AP lesions?
4. *Root-filled teeth without AP:* Should endodontically treated teeth without evidence of AP be considered as having had any contribution toward the subject’s total burden of endodontic inflammation?

There likely would be considerable disagreement in the endodontic and epidemiologic communities regarding how best to answer the above questions. To my knowledge, there is only one full-length, peer-reviewed report that specifically examines the relationship between endodontic disease and a systemic outcome, and it might be useful here to see how those

investigators the estimated burden of endodontic inflammation. Frisk et al. (78) reported no statistically significant relationship between the number of AP lesions and the presence of coronary heart disease in a cross-sectional investigation of 1056 Swedish women age 38–84 years. In that article, Method 2 (for cross-sectional studies) was used to classify burden of endodontic inflammation, as subjects were characterized as having 0, 1, 2, or > 2 AP lesions. Regarding the four questions above, in order:

1. No mention is made of lesion size, so one must assume that if a tooth was considered to have a lesion of any size, it was scored as a binary variable (yes/no).
2. No mention is made about lesions on roots versus lesions on teeth, but it is presumed that lesions were scored at the tooth level, so 32 would be the highest score any subject could have received.
3. AP lesions on endodontically treated teeth appear to have been scored identically to those on non-endodontically treated teeth.
4. Root-filled teeth were considered as a covariate in the multivariable logistic regression analysis. Each subject was categorized as having 0, 1, 2, or > 2 root-filled teeth.

Independence of observations

As mentioned earlier, many studies assess ‘endodontic success’ as the study outcome. In these papers, success generally is considered to be one of two outcomes, the other outcome being ‘failure’. The definition of success and failure varies among studies, but usually a successful case is one in which there is no AP at some defined time interval following RCT.

In general, any sample for which multiple observations per individual are assessed and analyzed should be considered a ‘cluster sample’ because the statistical assumption of independence among observations is violated (79). Prognostic endodontic studies are notorious for violating this principle, because not only can one individual contribute more than one tooth to a given analysis, but also multiple roots per tooth can potentially be examined, depending on the outcome assessed. A general rule is that if a dataset contains more than one observation per person, correlation among that individual’s outcomes are greater than if those same observations had been made on different people. This decreased variation leads to erroneously narrow

confidence intervals and increased Type I errors (i.e., finding statistical significance when no difference truly exists) (79).

One illustration of this problem is where an investigator wants to assess factors influencing endodontic success, and chooses the root as the unit of analysis, i.e., on multi-rooted teeth each root is assigned as having AP or not. It is quite reasonable that factors at different ‘levels’ might influence prognosis. In terms of AP, root-, tooth-, or subject-level variables exist. Root-level factors are those that are specific to a particular root that might influence AP on that root (e.g., degree of root curvature, presence of lateral canals, procedural factors such as apical perforation). Tooth-level factors would be those that could affect AP on roots through their effects on an entire tooth (e.g., coronal leakage of post-endodontic restorative treatment; overall skill of practitioner). Finally, ‘subject-level’ factors would be those pertaining to an individual subject that theoretically could affect all treated teeth in a similar fashion (e.g., subject’s immune or inflammatory status). One could imagine a study in which an investigator selectively followed all the endodontically treated teeth from one clinic during a period of time, but a single patient contributed six roots (e.g., two upper first molars). Because of tooth-level and patient-level factors common within that patient, the expected variation in success or failure would be expected to be less than that expected if six different individuals had been examined, each contributing one single-rooted tooth.

How can this problem be addressed? A biostatistician should be consulted initially to determine whether the study design incorporates a clustered sampling strategy. If it does, the investigator could:

- Create summary subject-level variables such as ‘proportion of filled roots that have AP’ and analyze the data with a conventional statistical software package. This technique is valid statistically but is accompanied by the loss of potentially valuable information at the tooth or root levels.
- Select only one unit of observation per person, e.g., one tooth or one root. The advantage of this strategy is that it effectively separates out each observation among study subjects so that all observations are independent of each other. The primary disadvantage is that there may be good information that would be easy to collect (especially

after a study subject is already present at the examination site) that would go uncollected. Another disadvantage is that the investigators must develop an algorithm to determine which tooth or root should be followed, e.g., the root with the worst initial PAI score, or the tooth treated first in chronological order. This choice may not be consistent across studies, leading to difficulty in comparing results.

- Use a software package that adjusts standard error estimates to account for clustered data within individuals. SUDAAN[®] (Research Triangle Institute, Research Triangle Park, NC, USA), and generalized estimating equations are among the methods that can be used for this purpose. The main advantage of this strategy is that these methods permit maximum use of the data, while the main disadvantage is that investigators must have access to the appropriate software packages and know how to use them properly.

Multivariable analysis

For many years, statistical analyses in the endodontic literature consisted primarily of bivariate assessment of relationships between various factors of interest and the primary study outcome. For example, Friedman et al. (80) and Smith et al. (68) present tables showing how pre-operative factors (e.g., tooth vitality, patient age), intraoperative factors (e.g., number of treatment sessions, extruded sealer), and/or post-operative factors (e.g., follow-up time, type of final restoration) related to results of endodontic therapy (classified as ‘success’, ‘incomplete healing’, or ‘failure’).

In bivariate analyses, factors of interest are compared on a one-by-one basis with the outcome, regardless of what other variables may be acting on the relationship. Bivariate analyses are certainly acceptable statistically and are a good first step in data analysis, but there are well-established methods for delving deeper into the data, primarily for the purposes of establishing how other ‘confounding’ variables might be acting on the observed relationships. Most notably, multivariable regression modeling can be used to more accurately calculate the strength of a relationship between an exposure and an outcome while taking into account the simultaneous effects of other variables.

Suppose an investigator designs a study to determine whether men with AP at baseline are more likely than

those without AP at baseline to develop CVD during the next 5 years. Further suppose that the study findings show that those with AP were indeed more likely than those without AP to have developed CVD; this would represent a bivariate analysis. However, it could be that those with AP were more likely than those without AP to be smokers, and because smoking is strongly related to development of CVD, any positive relationship observed between AP at baseline and CVD development might be in part or completely due to the fact that a confounding variable, namely smoking, was responsible for the observed relationship. Another example of interference from extraneous variables might be inflammation caused by periodontal disease, e.g., if the outcome of interest were a systemic biomarker of inflammation, it would be premature to infer that AP caused elevated levels of inflammatory biomarkers if inflammation from chronic periodontal disease were not measured and taken into consideration. Bivariate analyses, even a series of bivariate analyses, are unable to tease out the degree to which observed relationships of interest are affected by the presence of other variables. Multivariable regression modeling (e.g., linear, logistic, or proportional hazards regression, as well as special versions of those techniques that deal with clustered data) is a set of currently accepted and widely known methods used to ‘control for’ the effects of potential confounding variables and thus help improve confidence in statements about exposure-outcome associations. Examples of multivariable regression modeling in the endodontic literature can be found in Ray & Trope (60) and Kirkevang & Wenzel (54). Further discussion of this issue is beyond the scope of this manuscript, but it should be stated that at the present time, data analyses that stop at the bivariate analytic stage are inadequate, as better information can be obtained using more current methods.

Generalizability

For all epidemiologic studies, generalizability is desirable but not invariably attainable. Generalizability refers to the investigator’s or reader’s ability to transfer results obtained in one setting or population to the larger population of interest to him/her, which for clinicians usually is their own population of patients. A general rule is that readers should examine the age, sex, and socio-economic distributions of study subjects and

decide whether those subjects are representative of (i.e., similar to) their patient population. If so, conclusions made in the study might be applicable to that patient population.

Children most often are not included in studies of AP, simply because AP tends to be relatively rare in children but becomes more common with increasing age because of cumulative insults to teeth via caries and restorative therapy. The tables show that studies reporting the prevalence of AP and root-filled teeth generally do not include children, and in the relatively few instances where RCT is carried out in children it often results from an acute event like trauma rather than a bacterial infection of the dental pulp space. Fortunately, CVD and adverse pregnancy outcomes also are not outcomes that are generally studied in children, so populations studied with regard to AP and systemic outcomes likely all would be adult populations. It is well known that the chronic, cumulative nature of CVD manifests in disease more prominently among older age groups, and by definition, adverse pregnancy outcomes can only occur in women of childbearing age.

Selection bias

Selection bias is defined as ‘error because of systematic differences in characteristics between those who are selected for study and those who are not’ (37). In other words, it would not be appropriate to generalize results from any study to a population that includes individuals who would not have satisfied that study’s eligibility criteria. For example, AP cannot be assessed for teeth that are missing at the time of examination, so edentulous individuals must necessarily be excluded from participation in studies of AP, but there are examples in the AP prevalence literature where anyone with fewer than 10 teeth (66) or fewer than nine teeth (51) have been excluded. Because individuals who are missing many teeth likely had experienced substantial amounts of disease on their missing teeth, they might also have experienced substantial disease on their remaining teeth, and without explanation for excluding these individuals, it is likely that their exclusion underestimates the total amount of AP in the larger population. It is recommended that investigators explain the reason for any exclusion criterion that would not be obvious to the casual reader, and also to speculate on the possible effects those exclusions might

have on the authors' conclusions regarding the larger population.

Data quality issues

Quality and type of radiographs

Diagnosis of AP can be difficult to make even with good-quality radiographic images, so without a doubt, images of questionable or poor quality should not be used for research of this type. In clinical practice, intraoral periapical radiographs are used to determine the presence of AP. In epidemiologic studies, both orthopantomographic images and full-mouth radiographic series have been employed. The validity of radiographic examination via orthopantomographs has been compared with that of full-mouth radiographic series with respect to certain endodontic variables and found to be sufficient for detecting root-filled teeth and AP (81–84). In one of these studies, orthopantomographs and full-mouth radiographic series were compared from 75 randomly selected study participants who had both types of radiographs exposed at baseline (84). Using full-mouth radiographic series as the gold standard with respect to osteolytic periapical lesions, sensitivity was 82–86% for single rooted teeth and 94–95% for multirrooted teeth, depending on whether widened periodontal ligament space was included in the definition of AP; specificity was not reported and was not calculable from the figures given. In addition, mean marginal bone scores measured using a five-graded transparent ruler (85) supplied a correlation coefficient of 0.96. These findings led the authors to state that '... panoramic radiographs can be considered a useful tool in epidemiological studies of oral health' (84). Orthopantomographs certainly could be used in studies where the definition of AP includes a 'gross' or 'obvious' radiolucency of endodontic origin in the periradicular areas, but this restriction necessarily would lead to numerous false negative calls by the radiographic examiners, who would not be able to discern small lesions clearly from these images.

Reliability of radiographic interpretation

Both clinical experience and the scientific literature (86–89) indicate that examiners differ in their ability to perceive and quantify details from radiographic images,

thus training and calibration in radiographic interpretation are essential features of a properly designed study in which the presence of absence of AP is to be evaluated. The advent of digital imaging may indeed heighten the potential for variation in radiographic interpretation within and across examiners, because examiners potentially can modify contrast, brightness, and magnification settings that could affect their interpretations.

Masking (blinding)

The determination of AP necessarily requires viewing radiographic information around the periradicular regions of teeth. For many examiners, especially those with substantial clinical experience and some knowledge of potential risk factors for AP, it is exceptionally difficult to make calls about AP status without peripheral vision of the entire tooth, including the entire root system and coronal tooth structure. Even if the protocol restricts the examiner to making a call on a certain root, realistically it is quite difficult to 'block out' the status of the other roots apparent on that same radiograph. For most examiners, calls regarding a tooth's apical status realistically and unfortunately can be influenced by the quality of its coronal status (especially in obvious cases of defective coronal restorations) and the technical quality of RCT. In situations where this happens, associations between coronal and/or RCT quality could be biased in the direction of finding stronger associations between these exposures and AP. AP is more common among teeth with poor technical quality RCT, and also more likely if the tooth's coronal aspect is not sealed adequately (60, 90), and the percent of teeth judged as having 'inadequate' RCT is substantial in virtually all studies reporting these figures (see tables). Unfortunately, to blind radiographic examiners with respect to these variables would require isolating the crown and root canal systems of each tooth on each radiograph, which would be an extraordinarily time-consuming exercise. Such isolation cannot be expected in large-scale investigations of this type, so proper training of examiners is of the utmost importance in this regard. Nevertheless, the lack of masking leaves open the possibility of erroneously high correspondence between poor-quality coronal restorations, poor-quality RCT, and the presence of AP.

Response rates

The response rate for an epidemiologic study can be defined as ‘the number of completed or returned survey instruments (questionnaires, interviews, etc.) divided by the total number of persons who would have been surveyed if all had participated’ (37). In the context of AP studies, where radiographic data are necessary to ascertain the presence or absence of AP, the response rate would be the proportion of people for whom adequate radiographic information was obtained among all those for whom that information could have been obtained, had all eligible invited participants provided that information.

Although it is not necessarily true that bias will result if the response rate is low, in general, the lower the response rate for a given study, the greater the opportunity for *response bias* to occur. Response bias is defined as ‘systematic error because of difference in characteristics between those who choose to volunteer to participate in a study and those who do not’ (37). If non-responders are similar to responders with regard to characteristics of interest, then minimal bias will result. However, in general it is assumed that people who choose to participate in epidemiologic studies tend to be more educated, have better health, and have better attitudes toward health behaviors than those who choose not to participate (91), unless there are incentives for study participation. Although there is no agreed-upon guideline for ‘How high should the response rate be?’, a commonly followed guideline is that response rates of at least 70% should be attained for results to be generalized with confidence to the larger population of interest. In endodontic prevalence studies, especially those from community-based samples, response rates often are considerably less than this figure (50, 63).

Response rates can be even poorer in studies where the assessment of AP occurs longitudinally and thus depend on people returning for evaluation long after endodontic treatment has been completed. It is not unusual for such studies to involve people in pain, of limited socio-economic means, or in poor general health, all conditions that make it difficult to conduct multiple, longitudinal assessments on the same individuals. Further complicating the issue is if response rates are not equal across comparison groups, opening the door for even greater potential for bias. For example, a longitudinal study by Fouad & Burleson (92) investi-

gating the effect of diabetes on endodontic success was plagued by an overall response rate of 10%, which was comprised of a 26% response rate among diabetics and only a 9% response rate among non-diabetics (93). Poor response rates are not an indictment of any particular research effort, but it should be routine for authors to examine potential differences between responders and non-responders and to speculate on possible effects those differences might have on their conclusions.

Attrition (loss to follow-up of subjects and teeth)

In longitudinal epidemiologic studies, *attrition* refers to loss of study material over time. In dental studies, attrition can occur through the loss of subjects (e.g., because of death, illness, refusal to participate in follow-up studies) or teeth (i.e., because of extraction) during follow-up. The *healthy worker effect* is a term used to describe how, in longitudinal studies, summary measures of population health can improve over time simply because the relatively healthier subjects tend not to present for recall visits. In dental studies, the healthy worker effect not only applies to subjects, but also to teeth. Suppose at baseline a subject has 24 teeth, including three with AP, so 12.5% of that individual’s teeth would have AP. Then suppose at that subject’s next visit those three teeth had been extracted, perhaps because the AP had become symptomatic and the subject chose not to undergo RCT. The extent of that subject’s AP would have decreased to 0% not because his/her AP had been ‘cured’ by endodontic treatment, but merely because the affected teeth were no longer present. Here, disease progression would result in the subject actually appearing to have less disease. For the most part, researchers can make substantial efforts in their study protocols to prevent attrition of subjects, but can do nothing to prevent attrition of teeth. Still, investigators should at least speculate in their reports as to the potential effects of tooth or subject attrition on their findings.

Misclassification

In the context of epidemiology, misclassification is ‘the erroneous classification of an individual, a value, or an attribute into a category other than that to which it should be assigned’ (37). Misclassification can lead to bias, either toward the null value (i.e., toward showing no association between the exposure and outcome

variables) or away from it (i.e., toward showing a stronger association), depending on the quantity of misclassification within comparison groups (38). The potential for misclassification is present in any clinical epidemiologic investigation, but there is an especially abundant opportunity for misclassification in studies involving AP. Below are just some of the many potential reasons for misclassification of AP:

- *Delayed radiographic evidence of the inflammatory process:* Substantial destruction of apical bone, roughly 30% of volume, must occur before the human eye is capable of detecting change radiographically (94–96). This phenomenon would lead to false negative calls of AP, because many true cases of AP would be called negative by radiographic examiners.
- *Two-dimensional images of three-dimensional objects:* Radiolucent periapical lesions may be obscured by radio-opaque anatomic structures, including roots of other teeth or maxillary/mandibular tori, and as such could lead to false negative calls of AP. Additionally, radiolucent AP lesions may be subject to false negative calls if they are superimposed over other radiolucent structures such as the maxillary sinus or the mental foramen. Conversely, radiolucent structures such as the mental foramen could be responsible for false positive calls of AP on lower premolars with normal periapices.
- *Missing teeth:* No measurements of AP can be made from teeth missing at the time radiographs are taken, so no assignment of AP history can be made for them. In addition, missing teeth usually are not missing at random; compared with other teeth, they likely had more disease prior to being extracted than present teeth (97). In studies of caries and periodontal disease, alternative analytic strategies have been proposed to adjust whole-mouth scores to minimize bias introduced by missing teeth (98, 99). However, no such adjustment has been proposed for root-filled teeth. Root-filled teeth can be extracted for the same reasons as non-root-filled teeth, including chronic periodontal disease, non-restorable fracture, and trauma, but they also can be extracted secondary to adverse endodontic mishaps such as perforation and vertical root fracture (100). Thus, one would expect root-filled teeth to be lost at a greater rate than that of non-root-filled teeth,

and because the examiner cannot know the endodontic status of missing teeth, underestimation of AP history is likely.

- *Apical radiolucencies of non-endodontic origin:* Not all apical radiolucencies represent chronic AP, as periapical cysts, foreign body reactions (101), or scar tissue (102) may be responsible for apical radiolucencies in rare instances.
- *Endodontic therapy for reasons other than chronic AP:* While many RCTs are performed to treat chronic AP, many others are conducted to relieve acute pulpitis. Still others are carried out for prosthetic reasons on teeth with normal, vital pulps (100), or subsequent to trauma or developmental disturbances (103). Teeth treated for these reasons may never have had periapical inflammation, because bacterial invasion may not have reached the root apices.

Further, investigators must remember that in addition to AP, the dependent variables also have the potential to be misclassified. For example, CVD might be misclassified when hospital discharge data and death certificates are taken at face value (104–106).

Summary and implications

The current state of the literature into relationships between periodontal disease and certain adverse health outcomes shows that, in general, people with chronic periodontal disease tend to be more likely to experience these outcomes than people who do not. Whether these relationships are causal has yet to be agreed upon, and resolution of this point will have enormous impact on both the health of the public and relevant scientific research efforts. If future investigations show that endodontic disease has a deleterious effect on systemic outcomes like CVD or adverse pregnancy outcomes, public awareness of the importance of caries prevention would be seen in an entirely new light. Although many people know that deep cavities can lead to toothaches, how much more attention would be paid to oral health maintenance if the scientific community showed that CVD and adverse pregnancy outcomes could be avoided by implementing those same measures used to fight caries? Clearly there is much potential gain if this area of inquiry is explored thoroughly, especially if associations between endodontic disease and systemic outcomes are confirmed through consistent findings

across studies conducted among different populations. Armed with an adequate understanding of some of the important issues related to the conduct of epidemiologic investigations, plus proper planning, execution, and interpretation of findings, the endodontic community would be well positioned to help improve the oral and general health of the public.

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